Atopic Dermatitis and the Stratum Corneum

Part 1: The Role of Filaggrin in the Stratum Corneum Barrier and Atopic Skin

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Abstract

This three-part review presents what is currently known about the involvement and interdependency of the barrier properties of the epidermis, especially the stratum corneum and various specific immunological responses in the etiopathogenesis of atopic dermatitis. Part 1 of this review depicts the role of filaggrin in atopic dermatitis while Part 2 (which will be published in an upcoming issue of *The Journal of Clinical and Aesthetic Dermatology*) evaluates the role of serine proteases and specific lipids in the structural and functional integrity of the stratum corneum and related barrier functions in atopic dermatitis. Filaggrin is a key component of the stratum corneum that is derived from a larger precursor protein and contributes to its physical strength, hydration status, skin pH, and buffering capacity among other physiochemical properties. Filaggrin gene loss of function mutations appear to play a pathophysiological role; however, they are not the sole pathogenic factor in atopic dermatitis. Adverse structural changes of the stratum corneum are caused by upregulation of serine proteases activity, which causes degradation of certain stratum corneum proteins that are integral to barrier functions; interference with the formation of the stratum corneum intercellular lipid membrane, which normally regulates epidermal water flux and gradient; and induction of a TH2 pattern of inflammation, which is characteristic of atopic skin. Alteration in lipid ratios and changes in lipid-directed enzymes may play a role in the impairment of epidermal barrier functions that are associated with atopic dermatitis. Part 3 of this review (which will be published in an upcoming issue of *The Journal of Clinical and Aesthetic Dermatology*) discusses how immune dysregulation, including upregulation of a TH2 inflammation pattern, augmented allergic sensitization, sustained wound healing inflammation, and impaired innate immunity, all play a role in the development of atopic dermatitis. An increased understanding of the interdependence, polymorphisms, and dysregulations of epidermal barrier functions, including the stratum corneum permeability barrier, immune response barrier, and antimicrobial barrier, should provide further knowledge about the pathophysiological mechanisms that are related to the development of atopic dermatitis, are clinically relevant, and can better direct researchers to develop therapies that are targeted at important pathogenic components of the disease state.

Introduction

"Red and itchy," the classic findings that characterize atopic dermatitis (AD), are easy to describe, but the etiology of AD is a much more complicated matter. This common, often chronic and frequently frustrating, condition results from a complex interplay between abnormalities of several epidermal barrier functions, most of which primarily involve the stratum corneum (SC) and multiple immune defense mechanisms. As our understanding of disease mechanisms has increased, it is also very clear that no one defect can account for the array of clinical findings that can be associated with AD. Rather, the genesis of AD likely lies in the interaction between a genetically distinct immune response and impairments of the SC. Together,

these abnormalities, in the right environmental milieu, conspire to predispose the affected individual to hypersensitive responses to normal environmental elements and less capable of withstanding the "wear and tear" of daily environmental assaults on the epidermis.[1]

The latest scientific evidence on SC barrier disruption, which characterizes AD, as well as greater knowledge about the immune defects present in atopic individuals, has allowed for both a better understanding of the disease and more appropriate use of therapies. Although the structural and functional aberrations of the SC and the immunological abnormalities that are present in AD will be discussed separately, it is clear that both SC changes and immune dysregulation are interdependent in AD and are intertwined in creating the altered and impaired responses of skin to various exogenous challenges, including allergens, irritants, microbial organisms, and climatic changes.

Stratum Corneum Barrier Function and Atopic Dermatitis

The SC barrier is not an inert shell comprised of keratin fibrils, but rather is a metabolically active and physiologically responsive semipermeable membrane that functions as a biosensor to protect our body from untoward environmental elements and to maintain physiological water balance. This "force field," when functioning at full capacity, possesses the ability to "retain the good within" and to "keep the bad out" and to recognize the difference between the two. In other words, the epidermal barrier permits percutaneous absorption of water and other essential nutrients in the skin, prevents certain important substances in the skin from diffusing outward, and also blocks certain noxious substances and infectious organisms from gaining access to the inner more fragile layers of the skin.[2–5]

The SC is the major epidermal component of the so called "epidermal barrier" and is directly involved in several barrier functions.[2] There are several known abnormalities of the SC barrier that may contribute to the disruption of the epidermal barrier resulting in mechanisms that are operative in the pathophysiology of AD. These include defects in filaggrin,[6] increased serine protease activity,[1] and decreased ceramide fractions and total SC lipid levels.[7,8]

Filaggrin and the Stratum Corneum Barrier in Atopic Dermatitis

Filaggrin basics. Filaggrin is a key component of the epidermis that contributes to its physical strength as well as many other physicochemical properties. Filaggrin (filament-aggregating protein) was named by the late Peter Steinert, and its gene, FLG, is located in close proximity to the deoxyribonucleic acid (DNA) for many other protein products involved in the terminal differentiation of keratinocytes and in the epidermal differentiation complex on chromosome 1q21. The initial product of the FLG gene, profilaggrin, is a 400-kilodalton polyprotein consisting of 10 to 12 tandem repeats of the filaggrin peptide. Profilaggrin and filaggrin are the main components of the F-type keratohyalin granules of the epidermis and are responsible for the designation and characteristic appearance of the stratum granulosum. Upon the terminal differentiation of keratinocytes in the epidermis (i.e., progression from the stratum granulosum to the stratum corneum), profilaggrin is dephosphorylated and then proteolytically cleaved by the protease maltriptase into multiple copies of the 37-kilodalton filaggrin peptide. The dephosphorylation and proteolytic cleavage of profilaggrin to filaggrin is regulated by calcium ion concentration and the serine protease CAP1/Prss8 as well as the protease inhibitor lymphoepithelial Kazal-type trypsin inhibitor (LEKTI).[4–6,9]

The newly formed filaggrin peptide binds to the keratin cytoskeleton forming the filament-matrix complex, which also binds to the newly formed cornified envelope. This bond between the cytoskeleton keratin, filaggrin, and the cornified envelope collapses the keratinocytes and results in the flattened squames characteristic of the SC called corneocytes. Corneocytes are strongly anchored to one another by the keratin-filaggrin attachments of the filament-matrix complex, the crosslinking of the extracellular cornified envelope, and the desmosomal proteins anchoring the keratin filaments of neighboring corneocytes.[1,5]

Interestingly, the function of filaggrin changes as it courses up through the layers in the epidermis. In the inner layer of the SC, filaggrin contributes to the physical strength of the SC barrier through its integral involvement in the filament matrix complex as described above. Without filaggrin, the integrity and cohesion between corneocytes is weakened and becomes "leaky," allowing augmented percutaneous penetration and increased transepidermal water loss (TEWL) through the corneocytes.[1,4]

However, in the outer layers of the SC, filaggrin is degraded into free amino acids (AA), urocanic acid (UCA), and pyrrolidine carboxylic acid (PCA), which are collectively known as natural moisturizing factors (NMFs). NMFs are formed from the post-translational modification of the

c-terminal portion of filaggrin by caspase 14. NMFs are important to the function of the SC as they provide moisture retention (humectancy), maintain the acidic pH and buffering capacity of the SC, promote proper epidermal maturation and desquamation, and decrease pathogenic bacterial colonization.[1,10] The development of filaggrin and its degradation products is a delicate balance involving production, proteolysis, and inhibition of this process, with every intricate step of this needing to function to a specific degree in order to produce a healthy and effective SC barrier.

Filaggrin and skin hydration. Filaggrin plays an integral role in maintaining skin hydration by preserving SC integrity and the production of NMF. Filaggrin deficiency thus leads to compromised integrity of the SC and decreased levels of NMF and occurs in the presence of loss-of-functions mutations of FLG.[4–6,10] The compromise in SC integrity is reflected by an increase in TEWL and skin dehydration and a decrease in the levels of NMF, leading to an impaired ability for corneocytes to absorb and retain water at levels necessary for normal physiological function. Thus, deficiencies in filaggrin and NMF lead to dehydration of the upper layers of the SC. Decreased water content creates a steeper water gradient in the upper layers of the SC, which further exacerbates water loss and evaporation from the skin leading to further skin dessication. In addition, unchecked SC dehydration further amplifies a perpetuating cycle as a dehydrated local environment inhibits filaggrin proteolysis, which leads to further reduction in NMFs, followed by greater SC water deficiency, which produces greater compromise of SC enzyme function.[4]

The association between a deficiency of filaggrin and its breakdown products (NMFs) and the xerosis that is characteristic of AD was demonstrated by Kezic et al.[11] They confirmed that individuals who carry FLG-null mutations (i.e., have no filaggrin production) have significantly reduced levels of NMF in the SC on both the forearm and the palms at all SC depths; in addition, individual carriers of FLG mutations with a history of AD had significantly lower NMF levels than those with a history of AD who were noncarriers of FLG mutations ($P<0.0001$). They also demonstrated higher TEWL in the carriers of FLG mutations as compared with noncarriers.[11]

Other functions of NMFs. In addition to maintaining skin hydration by providing humectancy within the SC, NMFs have other important functions in the SC. These functions include maintaining skin pH and buffering capacity[12] and potentially decreasing colonization, and hence subsequent infection, by pathogenic bacteria.[13]

Alterations in baseline skin pH and buffering capacity in AD are partially due to deficiencies in NMF. In AD, baseline skin pH is more alkaline than the average baseline skin pH of healthy skin, which is 4.0 to 6.0. Urocanic acid breakdown products, free fatty acids, and sodium hydrogen exchange (NHE1) are the three main mechanisms responsible for maintaining an acidic skin pH. In AD, NMFs, including UCA, are deficient, explaining the increase in skin alkalinity. Clinically, it has been shown that higher skin pH in AD correlates with an increased severity of disease.[14]

Healthy skin has the ability to maintain an optimal acidic skin pH despite the application of alkaline substances due to its inherent buffering capacity.[12] Patients with AD are more susceptible to variations in pH following exposure to acidic and alkaline substances due to impairment of buffering capacity. It is thought that the buffering capacity of the SC is derived from free water soluble AA.[12]

These AA in the SC likely originate from the breakdown of filaggrin as AAs derived from filaggrin comprise approximately 40 percent of NMFs.[12] Since many patients with AD are deficient in filaggrin,[15] the ensuing decrease in AA appears to at least partially explain the impaired buffering capacity noted in atopic skin.

Maintaining skin pH in an acidic range and maintaining an inherent buffering capacity is important because increased SC baseline pH leads to increased activity of SC serine proteases (SP),[16,17] augmented susceptibility to infection,[12] reduced secretion of lipids,[8] and decreased function of lipid processing enzymes,[8,16] all of which are deleterious to normal skin barrier function.[8,16]

Under normal conditions, bacteria have to overcome acidic conditions and evade the detection and the defenses of the immune system in order to survive on the skin. Given that AD patients have abnormalities in skin pH in addition to inherent impairments of innate immune response (discussed in detail later), it is not surprising that *Staphylococcus aureus* can be isolated from lesions in 90 percent of adults with AD, whereas *S. aureus* is only detected in five percent of subjects with healthy skin.[18,19] *S. aureus* colonization in healthy skin is usually transient as inherent skin defenses normally do not allow *S. aureus* to sustain as part of the normal cutaneous flora. However, when SC barrier functions and immunological response function

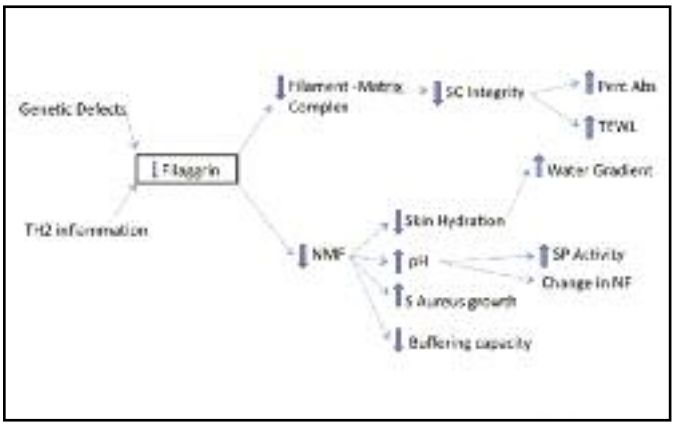

Figure 1. The effects of decreased filaggrin on the stratum corneum[1,4,12,16–17,20–21]

Key: NF=normal flora, NMF=natural moisturizing factor, Perc Abs=percutaneous absorption, S aureus=*Staphylococcal aureus*, SC=stratum corneum, SP=serine protease, TEWL=transepidermal water loss

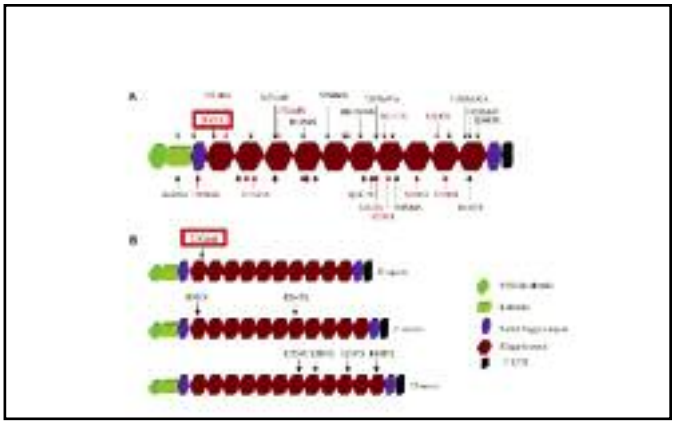

Figure 2. Location of mutations of the FLG gene. The mutations R501X and 2282del4 are highlighted in red. Reprinted with permission from O'Regan GM, Sandilands A, McLean WHI, Irvine AD. Filaggrin in atopic dermatitis. *J Allergy Clin Immunol.* 2008;122:689–693.[4]

suboptimally, pathogenic organisms, such as *S. aureus*, are opportunistic and gain access to colonize skin and sometimes induce infection. Given that null mutations in the FLG gene result in reduced SC integrity, increased percutaneous absorption, increased pH, and reduced levels of UCA and PCA,[2,20,21] it is thought that altered filaggrin may play a significant role in the increased colonization and infection from pathogens, such as *S. aureus*, in AD.

In order to substantiate the role of filaggrin and its breakdown products in the growth of *S. aureus* on skin, Miajlovic et al studied the growth of *S. aureus* in the presence of the filaggrin breakdown products UCA and PCA at concentrations similar to those found on healthy skin.[13] They found that the principal filaggrin breakdown products, UCA and PCA, exerted profound inhibitory effects on *S. aureus* proliferation, adhesion, and survival at physiological concentrations, and these effects lessened as UCA and PCA concentrations decreased. Miajlovic concludes that these results, although conducted *in vitro*, suggest a link between FLG gene mutations, NMF deficiencies, and increased *S. aureus* colonization and infection in AD.[13] The role of filaggrin and NMF in the SC in AD is summarized in Figure 1.

Specific FLG mutations in atopic dermatitis. Loss-of-function mutations in the FLG gene and the resultant filaggrin deficiency were first discovered in association with icthyosis vulgaris (IV) in 2006. Today, we are aware of 45 ancestral mutations in the FLG gene. FLG gene mutations are thought to be the strongest and most widely replicated genetic risk for AD.[1] Of all the filaggrin mutations that have been discovered to date, the two that are most commonly associated with AD are R501X and 2282de14.[4,20,21] Both R501X and 2282de14 FLG mutations are biochemically equivalent leading to a complete loss of FLG expression.[4,20,21]

In order to better understand the contribution of null mutations of the FLG gene to the risk of developing AD, Baurecht et al completed a meta-analysis of different studies investigating the odds ratio (OR) of developing AD in those patients with RX501 and 2282de14 FLG null mutations.[21] The results of this meta-analysis published in 2007 demonstrated a high risk of developing AD associated with both null FLG mutations, with an estimated overall odds ratio (OR) of 3.51, 3.62, and 3.58 for RX501, 2282de14, and the combined genotype, respectively.[20] Another similar meta-analysis was completed in 2009 by Rodriguez et al who also found a high risk of developing AD in those patients with both FLG null mutations, with an estimated overall OR of 3.14, 2.78, and 3.12 for R501X, 2282de14, and the combined genotype, respectively.[20] Both studies found that moderate-severe cases of AD had the highest association with FLG null mutations. The link between these specific FLG mutations and AD is higher than some of the strongest genetic links known to date, such as the OR for ADAM33 gene mutations and developing eczema or TCF7L2 gene mutations

and developing diabetes.[21] However, these estimates were obtained by pooling a number of heterogeneous study designs so these findings should be interpreted cautiously. Figure 2 identifies filaggrin mutations; the two most common are highlighted.

The role of filaggrin in atopic dermatitis. Given our current understanding of the SC barrier defects in AD and the association between FLG gene mutations and AD, it seems that a deficiency of filaggrin may be the unifying common denominator to explain the SC barrier insufficiencies seen in AD. The SC barrier defects associated with filaggrin deficiency in AD include diminished SC barrier strength/integrity, increased percutaneous absorption, increased dehydration, increased alkaline skin pH, decreased buffering capacity, increased SC protease activity, and decreased antimicrobial function.[1,2,4,12,15,22,23]

However, SC barrier defects from FLG null mutations may not be sufficient to induce the classic cutaneous findings of AD, given that patients with FLG mutations can develop IV without manifestations of AD, and 50 percent of AD patients do not have FLG defects.[15] However, patients with IV do have the propensity to develop AD later in life,[15,24] and these questions remain: Why do some people with FLG defects develop IV while others develop AD? And what changes within the skin spur the transition from filaggrin deficient IV to AD?

To address the above questions, Fallon et al conducted an experiment using flaky tail mice with a homozygous 5303delA mutation in the FLG gene. The 5303delA mutation is associated with loss of the C-terminal profilaggrin epitope and appearance of a 215 kDa truncated profilaggrin molecule in the skin of the flaky tail mice.[15] Since filaggrin and its degradation products are normally derived from the c-terminal of the profilaggrin molecule, the absence of the c-terminal of profilaggrin in flaky mice results in filaggrin and NMF deficiency, and thus presumably represents an appropriate model for human filaggrin deficiency. In his initial evaluations, Fallon noted that the filaggrin-deficient flaky tail mice at baseline demonstrated the phenotypic, histological, and ultrastructural characteristics of IV rather than AD.[25] Given this, Fallon et al sought to determine if exposure to allergens would induce changes in these filaggrin-deficient mice similar to AD. Fallon found that the exposure to allergens and resulting IgE-mediated allergic sensitization induced characteristic changes of AD in the flaky tail mice. In addition, following allergic sensitization, Fallon et al witnessed a worsened SC permeability barrier function confirmed by an elevation in TEWL. The findings of these experiments provide evidence that antigen transfer through a defective SC barrier is a key mechanism underlying elevated IgE sensitization and initiation of cutaneous inflammation associated with AD. While clarification of this relationship between FLG, IV, and AD warrants more experimentation, Fallon et al has demonstrated that allergic sensitization in addition to an inherited SC barrier defect, such as FLG mutation, may explain the mechanism of transition from a filaggrin-deficient patient with IV skin changes to a filaggrin-deficient patient with AD.[15]

While associations between the FLG gene, AD, and the SC barrier have provided us with a scientifically plausible pathogenic explanation for many of the clinical manifestations of AD, there are many other aberrations of the SC in AD other than filaggrin defects that may also result in SC barrier dysfunction and increased potential for allergic sensitization. These SC defects include decrease in ceramide subfractions and total lipids, altered physiological lipid rations, and excessive serine protease activity.[7,8,17] Regardless of the origin of the barrier defect, all roads lead to decreased structural and functional integrity of the SC, and therefore an increase in exposure to potential antigens, allowing for allergic sensitization and the potential for AD skin disease.

Evolutionary advantage to FLG defects. It has been proposed that FLG gene mutations and/or filaggrin deficiency may be an evolutionary adaptive advantage in Western civilization. This adaptive advantage may be somewhat explained by the "hygiene hypothesis" of AD. The hypothesis attempts to explain the finding that children growing up in Western civilization with less exposure to infectious agents and allergens were more prone to developing allergies later in life compared to children growing up in developing countries with frequent exposure to infectious agents since birth. It is thought that barrier defects caused by FLG mutations advantageously allows for increased allergen and infectious exposure in children raised in Western civilization at an early age resulting in a relatively higher Th1 response, with dampening effect on the Th2 response that is overactive in atopic individuals. In essence, the filaggrin-associated weakening of the SC barrier promotes a "vaccination-type response" at an early age to these pathogens. Therefore, the increased exposure to the allergens and pathogens secondary to the SC barrier defects could have been advantageous at an early age in Western civilization

explaining the possible evolutionary advantage of FLG defects and other epidermal barrier dysfunctions.[6,26]

Conclusion

A deficiency in filaggrin leads to a reduction in SC hydration as the production of filaggrin-generated NMFs is decreased. Insufficiencies in filaggrin also change the pH and buffering capacity of the skin. These structural and functional changes to the epidermis lead to many downstream alterations in skin function and health. As important as filaggrin is to skin health and as strong as its genetic association to AD may be, lack of filaggrin due to genetic mutation is not the sole pathogenic factor that can explain the entire pathophysiology of AD, which is far more complex.

In Part 2 of this three-part review (which will be published in an upcoming issue of *The Journal of Clinical and Aesthetic Dermatology*), other innate epidermal impairments, such as increased serine protease activity and altered lipid ratios that contribute to the pathophysiology of AD, are discussed. These abberations predispose the patient to exacerbation of xerotic and eczematous skin changes. The net effects of these changes, including changes in filaggrin, are a reduced ability of the SC in atopic skin to self-repair when challenged by exogenous insults, with prolonged signaling of inflammatory cascades leading to exacerbation and worsening of xerotic and eczematous skin changes.

Part 3 of this review (which will be published in an upcoming issue of *The Journal of Clinical and Aesthetic Dermatology*) discusses how immune dysregulation, including upregulation of a TH2 inflammation pattern, augmented allergic sensitization, sustained wound healing inflammation, and impaired innate immunity, all play a role in the development of AD.

An increased understanding of the interdependence, polymorphisms, and dysregulations of epidermal barrier functions, including the SC permeability barrier, immune defense barrier, and antimicrobial barriers, should provide further knowledge about the pathogenic mechanisms that cause AD that is clinically relevant and leads to development of targeted therapies.

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SKIN STRUCTURE AND FUNCTION